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THE LIFE CYCLES, INTERMEDIATE HOSTS, AND LARVAL STAGES OF *RHIPIDOCOTYLE TRANSVERSALE* CHANDLER, 1935 AND *RHIPIDOCOTYLE LINTONI* HOPKINS, 1954: LIFE-CYCLES AND SYSTEMATICS OF BUCEPHALID TREMATODES[1]

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Rhipidocotyle transversale was described by Chandler (1935) from metacercariae encysted in the muscles of silverside minnows, *Menidia menidia*, from Galveston Bay, Texas. *Rhipidocotyle lintoni* was described by Hopkins (1954) from mature specimens found in the intestine of gars, *Strongylura marina*, taken at Port Aransas, Texas, Grand Isle, Louisiana, and Gloucester Point, Virginia. These worms have been known for seventy-five years, but intially the species were confused, the descriptions were deficient, and the taxonomic allocations were erroneous. They had been included in the genus *Gasterostomum* by Linton in earlier publications.

Linton (1900) described and figured unidentified gasterostome trematodes from *Tylosurus marinus* (= *Strongylura marina*) and juvenile specimens encysted in *Menidia notata* at Woods Hole, Massachusetts. During the summers of 1901 and 1902, he studied parasites of fishes at the laboratory of the U. S. Bureau of Fisheries in Beaufort, North Carolina. Notes and sketches made there provided the basis for his report (Linton, 1905), in which he described gasterostomes from the intestines of a number of fishes under the name *Gasterostomum gracilescens* Rudolphi. Encysted metacercariae from *M. menidia* were assigned to the same species. The specific description from the 1901 notes reads, (p. 357), "numerous; fusiform, pale red to colorless; not much variation in size. One measured 1.4 mm in length and 0.56 mm in greatest breadth." The 1902 notation recorded, (p. 357), "Dimensions, life, compressed; length, 1.4; maximum breadth 0.65; transverse diameter, anterior sucker 0.33; mouth 0.10; ova 0.018 by 0.011." "Small, minutely spinose, of very variable shapes, translucent white to orange yellow, depending on relative abundance of ova. *The anterior end in some was curved ventrad, turning the sucker into a hood-like organ*" (italics added). After describing specimens from *S. marina*, Linton (1905) noted, p. 357, "This same species was found in this host at Woods Hole, Mass., but was not identified. (Bull. U. S. Fish Commission for 1899, pp. 277, 298, pl. XLI, Fig. 91, and p. 442, pl. XXXIV, Figs. 367, 368)". Figures 91 and 368 show an expanded lobe overhanging the sucker and can be identified as *R. transversale*. Linton (1905) reported *G. gracilescens* from other fishes; encysted metacercariae in the muscles of *M. menidia* and on the viscera of the striped anchovy, *Stolephorus brownii*;

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immature forms from the intestine of *Opsanus tau* and *Caranx hippos;* and mature worms from *Paralichthys albiguttatus* and *Pomatomus saltatrix.* The metacercarciae were almost full-grown in *M. menidia* and it is possible, indeed probable, that the specimens from hosts other than *S. marina,* may be the result of predation.

Gasterostome trematodes were reported from fishes of Bermuda by Linton (1907) and of Tortugas, Florida (Linton, 1910), but without significant new information. The descriptions are inadequate and the generic allocations are equivocal. A summary and review of his studies on trematodes of fishes mainly from the Woods Hole region was published by Linton (1940). In it, the species formerly described as *G. gracilescens* was transferred to *Prosorhynchus.* The specific description was revised, augmented, and illustrated by figures 245–249. Linton noted (p. 31), "In some there is a distinct cap overhanging the anterior sucker (pl. 18, Fig. 246). In others the cap is reduced to a buttonlike process, and in still others there is no trace of it. In some, also, the anterior sucker appears to be retracted (pl. 18, Fig. 247)."

Hopkins (1954) reported that the gasterostome trematodes from *S. marina,* described by Linton (1900, 1905, 1940) as *Proserhynchus gracilescens,* belong to two different species; one is identical with *Rhipidocotyle transversale* Chandler, 1935 and the other was described as a new species, *Rhipidoctyle lintoni.* The species with the hood-like lobe overhanging the anterior sucker was recognized as *R. transversale,* while *R. lintoni* had only a button-like lobe. Hopkins found gars, *S. marina,* infected with both species and *M. menidia* carrying metacercariae of *R. transversale* at Port Aransas, Texas; Grand Isle, Louisiana; and Gloucester Point, Virginia. Figures 245 and 248 in Linton (1940) represent specimens of *R. lintoni* and Figure 246 is of *R. transversale.* It is clear that Linton had both *R. transversale* and *R. lintoni,* and representatives of the two species were included in his description of *P. gracilescens.*

Linton (1900, 1905) had indicated the probable identity of the metacercariae encysted in *M. menidia* and the mature worms from the intestine of gars. This determination was confirmed by Hopkins (1954); he predicated that the metacercariae are specifically identical with *R. transversale,* but the metacercariae of *R. lintoni* were not recognized. Adults of both *R. transversale* and *R. lintoni* occur regularly in *S. marina* during the summer at Woods Hole and metacercariae (juveniles) of both species are common in *M. menidia* throughout the year. Both species may occur in one and the same fish, adults in gars and metacercariae in silversides. In distribution, the two species, *S. marina* and *M. menidia* extend from Cape Cod to Texas. So far as it is known, gasterostome trematodes utilize bivalve mollusks as first intermediate hosts. Accordingly, it is clear that certain bivalve species, which has the same distribution and occur in the same ecological milieu as the gars and silversides, must harbor the asexual generations of *R. transversale* and *R. lintoni.* A list of such mollusks was prepared and a systematic search was undertaken. The more common species, especially those of economic importance that have been extensively studied, could be excluded and efforts were concentrated on the small, inconspicuous clams. This procedure led to the discovery that *Lyonsia hyalina* harbors the larval stages of either or both *R. transversale* and *R. lintoni.* In a preliminary note, Stunkard (1974b) announced the first intermediate host.

The adult stages of *R. transversale* and *R. lintoni* occur in the same host-species, and both were described briefly by Hopkins (1954). The metacercariae of both develop in *M. menidia,* and it is probable that the sporocyst and cercarial generations of both species occur in *L. hyalina,* but present information is not adequate to distinguish between them. The sporocysts and cercariae do not manifest morphological features that can be used for specific determination and it is probable that biochemical methods must be employed to decide whether *L. hyalina* harbors one or two distinct species.

Material and Methods

Strongylura marina is a littoral species that frequently ascends rivers to considerable distances. It feeds on small fishes, especially *M. menidia.* According to Breder (1929), it is "known from Maine to Texas, but not common north of Cape Cod."

Menidia menidia is omnivorous; the fishes congregate in schools of similar-sized individuals. They frequent sandy and gravelly shores among clumps of sedge-grass (*Spartina* sp.) in inner bays and river-mouths and follow the tides a few meters from the water's edge. They are resident throughout the year and often run into brackish water. Formerly, two species of *Menidia* were recognized; *M. notata* in the area from Nova Scotia to Virginia and *M. menidia,* from Virginia to Texas; but the populations intergrade and specific distinctions are no longer acknowledged. The second intermediate host, *M. menidia,* carries natural infections of *R. transversale* and *R. lintoni* and provides developmental stages of both species, from recently penetrated metacercariae to encysted, full-grown juveniles. However, metacercariae of the two species were recognized only after development of the lobe overhanging the anterior sucker. This feature is difficult to evaluate since the lobe is muscular, mobile, and may appear and disappear with contractions of different sets of muscles.

Experimental infection of *M. menidia* was effected; large and small specimens were exposed to cercariae and later examination of the fishes disclosed details of penetration by the bodies of the cercariae after the tails had been discarded. A fish was exposed in a bowl at 10 AM, July 27, 1974, to several hundred cercariae. It was removed at noon and very few cercariae were left in the water, while hundreds of tails were found on the bottom of the bowl. Fishes exposed to massive infections died in 24 to 48 hours. Other fishes, exposed to large numbers of cercariae, were transferred to small holding tanks and fed bits of clams, *Mytilus edulis* and *Modiolus modiolus.* But *M. menidia* is an active species and difficult to maintain in captivity. Many of the fishes died, but dissection of those that survived for two to four weeks provided small worms of experimental infection that were identical with those of natural infection.

Lyonsia hyalina is the first intermediate host of either or both *R. transversale* and *R. lintoni.* Observations on living bivalves of New England, including *L. hyalina,* were recorded by Morse (1919); their morphology and ciliary mechanisms were described by Kellogg (1915); and the development of *L. hyalina* by Chanley and Castagna (1966). Individuals reach a length of 16 to 20 mm; the shell is thin, fragile, and the periostracum bears fine, filamentous processes which collect and hold a coat of fine sand grains. The edges of the mantle are fused ventrally; the siphons are separated, very short, and surrounded by rows of small, sensitive tentacles. The animals lie buried, with only the posterior end exposed and the siphons are retracted on the slightest stimulation. They occur in somewhat muddy rather than sandy bottom. Infections are common in certain areas and absent in others that superficially appear similar.

The clams were isolated, 10 in each bowl, with the water changed twice daily, to find those that were liberating cercariae. When cercariae appeared in a bowl, the clams were isolated singly to find the shedder. By changing the water periodically, the time of cercarial emergence could be detected. The cercariae are very small, transparent, hard to see, and the addition of a trace of neutral red to the sea water was helpful, since the stain accumulated in the glandular cells and intestines of the cercariae, and the color enabled an observer to pick them up more readily. The cercariae emerged intermittently during the day and the night, sometimes more heavily at one time than another, and it appears that light does not affect emergence. Data on incidence of infection may be misleading, since it may be 5% in one location and virtually absent not far away. As a rule, specimens less than 10 cm long are seldom infected and incidence increases with size and probable age of the mollusks. Most of the infected clams were taken from Bourne Pond, near Falmouth, Massachusetts. Dissection often disclosed mature infections in specimens from which no cercariae were released. To study sporocysts and developing cercariae, clams were dissected; the digestive glands and gonads were studied alive, after fixation and staining, and when cut in serial sections 10 microns in thickness.

When placed in bowls of sea water, specimens of *R. transversale* and *R. lintoni* shed enormous numbers of eggs. Eggs of the two species are the same size, 18 to 20 by 11 to 12 microns. Those in the terminal part of the uterus are embryonated when passed; the shells are thick, yellow in color and so opaque that details of the miracidium are not distinct. No ciliary movement was observed, but an occasional twitching of the larva was noted. The eggs are operculate but did not hatch in sea water although kept under observation for more than three weeks at summer temperature. Attempts to obtain miracidia by pressure on a coverglass or by changed osmotic conditions were not successful. The miracidium is quiescent and probably can live for a long time under favorable conditions. This feature must have survival value; if the larva emerged soon after liberation of the egg, its brief, ephemeral existence would afford slight change of entering the inhalent siphon of *L. hyalina.*

Attempts to infect *L. hyalina* by dropping eggs into the inhalent spihon were not productive. The clams were planted in mud in petri-dishes with the siphons exposed and placed in a current of slowly running sea water. Eggs were dropped in the water above the siphons; if the tentacles around the siphons were stimulated, they closed immediately and the eggs were lost in the excurrent streams from the dorsal siphon. It is doubtful whether or not any of the eggs actually entered the mantle cavity. Other clams were embedded in mud in a large, shallow, flat dish, covered with a coarse screen and left for three weeks in the bottom of a tank which contained eight *S. marina.* All the fishes yielded mature specimens of either or both *R. transversale* and *R. lintoni* when autopsied at the end of the summer. Three egg-shells with open opercula were found in the feces of clams that had been exposed to eggs, but primary sporocysts were not found on dissection of the clams two or three weeks later. Indeed, since the clams were recently collected, it would have been impossible to determine whether or not recent infections were natural or experimental unless massive infections were obtained. Since the eggs do not hatch in sea water, infection must be accomplished by ingestion of eggs and emergence of miracidia in the digestive tract of the mollusk. The primary sporocyst may remain in the tissue of the digestive gland, but recent infections, consisting of slender branches with small germ-balls but no cercariae, are present chiefly in the gonad. Growth and development of the sporocyst generations progressively destroy the gonad and then invade the digestive gland. Eventually, all of the host tissues become depleted and survival is doubtful.

Adult worms were studied alive and after fixation and staining. In small bowls, live gravid specimens of *R. transversale* and *R. lintoni* manifest differences in appearance and behavior. The anterior end of *R. transversale* is sticky and bits of debris adhere to it, but this condition was not observed with *R. lintoni*. *Rhididocotyle lintoni* is more active; the worms migrated toward the edges of the bowl, whereas specimens of *R. transversale* remained near the center of the container. To obtain representative specimens, worms were fixed under slight coverglass pressure and good results were obtained also by the shaking method of Looss. With this method there is no flattening of specimens. In some, the genital atrium was everted with the protrusion of the genital lobes through the pore.

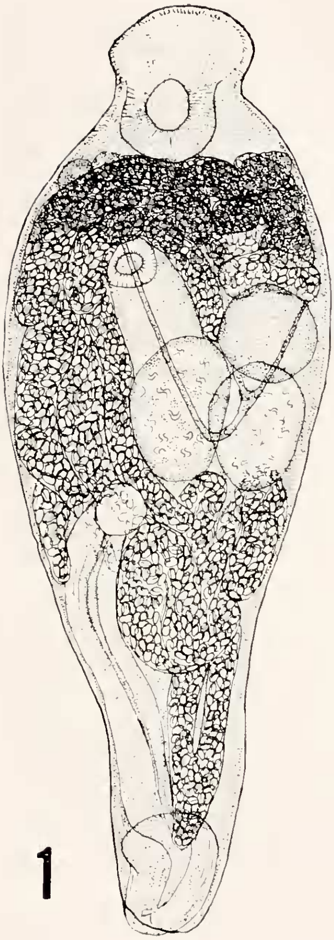
Existing descriptions of mature stages of *R. transversale* and *R. lintoni* are brief, incomplete, and present an unfortunate concept of the two species. They suggest a rigid, stereotyped structure; whereas, like all digenetic trematodes, the bucephalids are flexible, mobile, and highly variable worms. They manifest major changes in form and position of internal structures as different sets of muscles are contracted and relaxed. Mature worms from *S. marina,* when extended, may be narrow with almost parallel sides and three times as long as when contracted and expanded medially. With extension and retraction, there are corresponding changes in the relations of internal organs; the cirrus-sac may be some distance posterior to the gonads or almost contiguous with the anterior sucker, and there are resulting shifts in the arrangement of the coils of the uterus.

Examination of live material was facilitated by the use of vital dyes, especially neutral red. For permanent mounts, specimens were fixed in AFAG (alcohol, formalin, acetic acid, glycerol) or the solution of Duboscq-Brasil. Whole-mount preparations were stained with Mayer's paracarmine, Semichon's acetic carmine, and Ehrlich's acid haematoxylin. Sections were cut 10 microns in thickness which aids in computing measurements, stained with haematoxylin, and counterstained with erythrosin.

DESCRIPTIONS

Rhipidocotyle transversale (*adult stage*)

The description given by Hopkins (1954) is confirmed with additional information, especially on the reproductive systems. Fixed and stained gravid specimens (Fig. 1) measure 0.65 to 1.6 mm in length and 0.35 to 0.70 mm in width. Small specimens from the intestine of *S. marina,* 0.65 mm long and 0.35 mm wide may be



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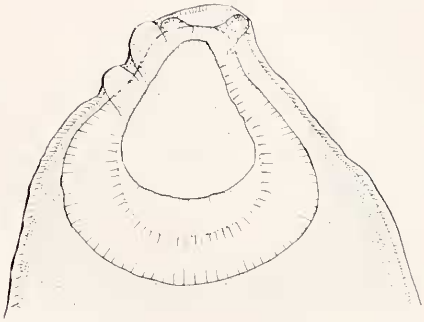

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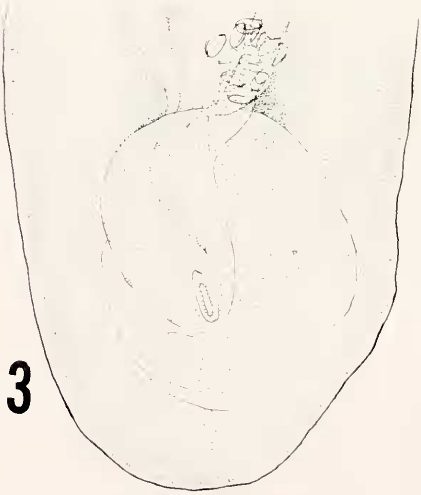

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FIGURE 1. *R. transversale*, dorsal view of 1.38 mm long gravid specimen, fixed after shaking, attenuate posteriorly, genital atrium partially protruded.

FIGURE 2. *R. transversale*, ventral view, fixed under coverglass, showing anterior papillae.

FIGURE 3. *R. lintoni*, ventral view of genital atrium.

gravid while others that are much larger do not contain eggs. The worms may become virtually full-grown as metacercariae, and it appears that if small individuals are ingested, they may become mature in the final host without reaching full size. Although bilaterally symmetrical, the body is somewhat cylindrical, almost circular in cross-section. Typically, the worms are oval to ovate, wider anteriorly and often attenuate posteriorly. The hood which surmounts the anterior sucker is a mobile, muscular organ. Contracted, it may be almost spherical, but in flattened specimens it is oval, 0.15 to 0.22 mm wide and 0.08 to 0.12 mm long. The hood is variable in form; in living worms the anterior edge may be rounded and firm or it may manifest small papillae (Fig. 2), probably five in number, but not all are visible at the same time. Ducts from some 20 or more glandular cells, located in the area around and posterior to the sucker, pass forward and open at the anterior tip of the body. The contents of the ducts stain intensely with Semichon's technique. The secretion probably accounts for the adhesion of particles to the anterior end of living worms.

The tegument contains minute, closely set spines which decrease in size and number posteriorly. The body wall consists of circular, longitudinal and oblique muscle fibers, and the parenchyma is relatively loose and vacuolate.

The mouth is median, ventral, located near the posterior end of the anterior third of the body. The pharynx is spherical to barrel-shaped, usually flattened, 0.09 to 0.11 mm in diameter. There is a short esophagus, surrounded by glandular cells. The intestine is saccate, often with one or more peripheral pockets, and its dimensions depend on the amount of contained material.

The excretory pore is terminal and the vesicle extends forward on the dorsal side of the body to the level of the gonads. The collecting ducts, capillaries and flame-cells are obscured in gravid worms but may be traced in juvenile specimens. The collecting ducts open near the anterior end of the vesicle, and the flame-cell formula is $2[(2+2)+(2+2)]$, as described by Hopkins (1954).

Male reproductive organs. The testes are spherical to oval, 0.15 to 0.25 mm in diameter. They are situated obliquely on the right side, near or slightly posterior to midbody length, and may partially overlap each other and the ovary. Their positions vary from tandem to opposite with changes in the elongation and retraction of the body. In the specimen shown in Figure 1, the anterior testis is ventral in position, slightly mediad and posterior to the ovary. The posterior testis is dorsal and partially overlaps both the ovary and anterior testis. Usually the posterior testis is posterior to the ovary. In other specimens, the anterior testis is dorsal and the posterior testis is ventral in position. Sperm ducts arise from the medial faces of the testes and unite to form the vas deferens, which opens into the anterior end of a long, cylindrical organ, usually described as a cirrus-sac. The designation is questionable since the organ does not enclose a cirrus and subserves other functions. It is located on the left side of the body about midway between dorsal and ventral surfaces and extends from the level of the gonads to the genital atrium. It may be bent or slightly sigmoid and, in Figure 1 it is 0.38 mm long and 0.12 mm wide. The wall is composed of an external layer of strong longitudinal fibers, inserted near the ends of the sac, and an inner layer of delicate circular fibers. In the anterior end, the vas deferens expands to form an oval to spherical seminal vesicle, 0.09 to 0.12 mm in diameter. From the vesicle a short curved duct opens into a long canal, surrounded by cells of the prostate gland, which extends posteriad through the cirrus-sac. It enters the ventrolateral aspect of the genital atrium where it becomes a thin-walled ejaculatory duct that opens into the cavity of the atrium.

The distal end of the "cirrus-sac" is inserted in a circular manner on the anterior wall of the genital atrium, (Fig. 3), an oval to pyriform organ, 0.15 to

0.20 mm in diameter which receives the ejaculatory duct from the prostate gland and the metratermal end of the uterus. The wall of the atrium is composed of longitudinal and strong circular muscle fibers. From the atrium, a short duct leads to the gential pore, situated ventrally near the posterior end of the body. From the anterior wall of the atrium two unequal, curved genital lobes extend backward and partially surround a cavity which is continuous with the duct to the external pore. In the posterior wall of the atrium there is a cluster of glandular cells. In certain worms killed by the shaking method of Looss, the genital atrium is everted and the lobes are protruded through the genital pore. In Figure 1, one of the lobes extends slightly beyond the posterior end of the body. Odhner (1905) described the male organs of *Prosorhynchus squamatus* and other gasterostomes and their probable function. He suggested that the glandular cells in the atrium of *Prosorhynchus* spp. produce a secretion that encloses spermatozoa to form spermatophores. In *Gasterostomum* spp., he postulated that contraction of the powerful longitudinal muscles of the wall of the "cirrus-sac" could exert pressure on the atrium, causing distension of the genital lobes and their protrusion through the genital pore and transmission of spermatozoa. He stated, p. 303, "Dass der Geschlechtszapfen hier als Kopulationsorgan fungiert, ist also ganz deutlich; bei *Gast. gracilescens* (Rud.) habe ich auch mehrmals beobachtet, dass das Organ aus der äusseren Geschlechtsöffnung hinausgragt."

Female reproductive organs. The ovary is spherical to oval, 0.12 to 0.20 mm in diameter, situated dorsally on the right side, near the middle of body-length. The oviduct arises from the median posterior face; it passes posteriad and ventrad and shortly gives off the Laurer's canal which opens on the dorsal surface of the body. Where the canal joins the oviduct, there may be a slight enlargement filled with spermatozoa, but a definite seminal receptacle is not present. The oviduct then receives the duct from the vitelline receptacle and enters Mehlis' gland. The gland is situated in the interval between the ovary and the testes and is oval, 0.06 to 0.08 mm in diameter. The vitellaria consist of a band of follicles, each 0.032 to 0.046 mm in diameter, which extends across the dorsal side of the body a short distance posterior to the anterior sucker. The follicles of the two sides meet in the median plane; short ducts from the individual follicles unite to form two larger ducts that pass laterad on either side and at the ends of the follicles pass posteriad. The vitelline ducts unite to form a loop which passes posteriad to the level of the testes and gives off a short common vitelline duct that expands into the reservoir and communicates with the oviduct as noted previously. The uterus emerges from Mehlis' gland and makes a loop posteriad, then turns forward and passes to the level of the vitelline follicles where it crosses to the opposite side of the body. The uterus continues in loops and coils, filling all available space anterior and posterior to the digestive cecum. The final descending limb ends in a short metraterm which enters the dorsolateral face of the genital atrium and opens into the cavity of the atrium. Eggs in the initial part of the uterus are thin-shelled, flexible in shape. The shells are permeable and young embryos stain readily. In later portions of the uterus the eggs become hard-shelled, yellow in color and are not permeable to stains. Eggs measure 0.018 to 0.020 by 0.011 to 0.012 mm.

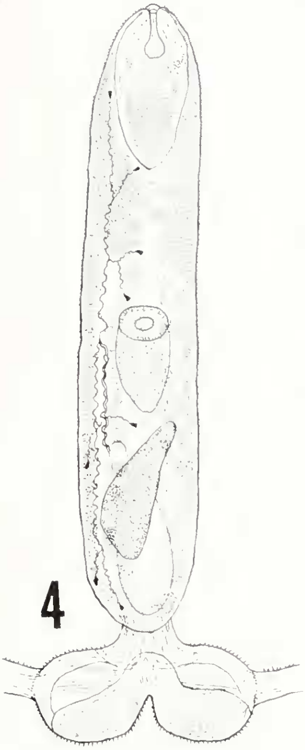
Rhipidocotyle lintoni (*adult stage*)

In the original description, Hopkins (1954) reported that *R. lintoni* is slightly smaller than *R. transversale* but that sizes of worms and of organs of the two species overlapped. The gland cells, so conspicuous around the anterior sucker of *R. transversale,* were not mentioned in the description of *R. lintoni.* The large hood or cowl, that surmounts the anterior sucker in *R. transversale* is represented in *R. lintoni* by a small cap. Hopkins noted differences in the shape and size of eggs of the two species; those of *R. lintoni* were slightly longer and narrower. His figure of *R. lintoni* shows a specimen with few eggs in the uterus, presumably a young worm, not fully mature.

Specimens of *R. transversale* and *R. lintoni* were abundant in the intestine of *S. marina* at Woods Hole, Massachusetts. Many mature *R. lintoni* are no larger than those reported by Hopkins; others are fully as large as those of *R. transversale.* The observations of Hopkins are confirmed, except for the size and shape of eggs. Examination of large numbers of eggs from *R. transversale* and *R. lintoni* did not reveal actual differences in shape or size. Except for differences in the hood and the absence of gland cells in the previtelline area of *R. lintoni,* the two species are so similar that a detailed description of *R. lintoni* would be a mere repetition of that of *R. transversale.*

Metacercariae

When the furcae of the cercaria make contact with a fish, they adhere firmly and their contraction brings the base of the tail-stem against the surface of the fish. Attachment by the base of the tail-stem provides a fulcrum from which the body of the cercaria attempts to enter the tissue of the host. The cercaria penetrates at the location of attachment. The cercariae will probably attack many kinds of fishes, but penetration and development apparently occur only in *M. menidia.* They may enter the gills, the wall of the mouth, around the eyes, and especially at the bases of the fins. On entry of the cercaria, the tail-stem and furcae are discarded and the body migrates by contraction of muscles in the body-wall and through vascular channels of the host. The cystogenous material was extruded early and after 24 to 48 hours, many of the worms were encysted. The worms encyst in muscles, even muscles to the eye-ball. The cyst-walls are thin, membranous and flexible. There was no visible reaction on the part of the host and no deposition of a second layer on the wall of the cyst. Immediately after encystment, the worms are smaller than the cercariae and do not completely fill the cysts which are oval to elongate. The cysts increase in size with development of the metacercariae and the largest measure 1.00 mm long and 0.50 mm wide. Since the metacercariae grow in their cysts, they must receive nourishment through the cyst-wall. Worms recovered two weeks after exposure showed little real growth. The intestine was enlarged and filled with oil droplets and cells of host-tissue, so the worms appeared larger and the largest, under cover-glass pressure measured 0.30 long and 0.09 mm wide. As noted, fish that received very heavy infections died in a day or two and most of the others were lost in the first two weeks. No specific differences were noted in the cercariae or in small metacercariae recovered four to six weeks after exposure. When the meta-



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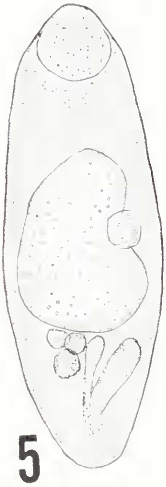

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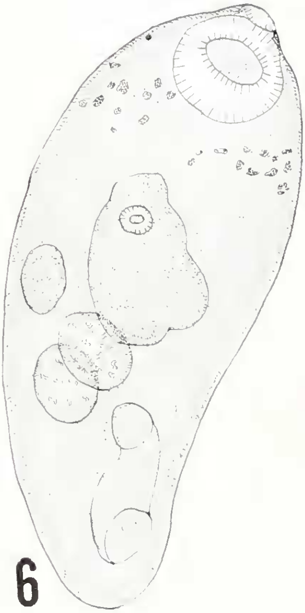

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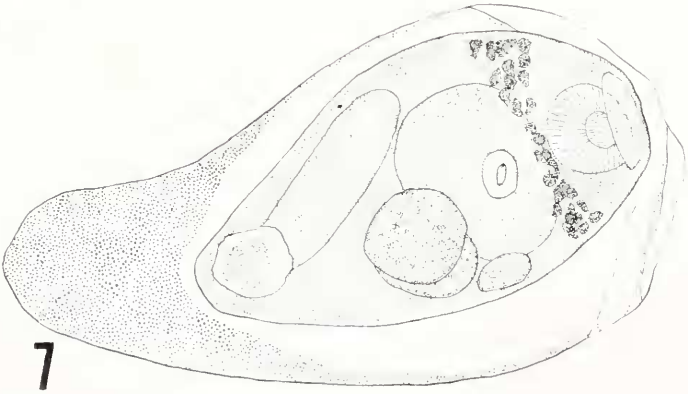

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FIGURE 4. *Rhipidocotyle* sp., Cercaria from *L. hyalina*, body and tail-stem (0.2 mm long); outline from fixed and stained specimen, details from living cercariae.

FIGURE 5. *Rhipidocotyle* sp., metacercaria at indifferent stage: shows regression of penetrantorium and formation of anterior sucker.

FIGURE 6. *R. lintoni*, metacercaria (alive, 0.5 mm), with early development of the vitellaria.

FIGURE 7. *R. transversale*, dorsal view, in cyst (1 mm long), vitellaria well formed.

cercaria is one-third to one-half grown, the anterior penetrating organ, designated the penetrantorium by Stunkard (1973) is undergoing regression and the anterior sucker is recognizable (Fig. 5). Only after transformation of the anterior ends and formation of the definitive lobes that surmount the anterior suckers can the two species be distinguished (Figs. 6 and 7).

Sporocysts

The sporocysts form tangled masses of colorless branching tubules that invade the gonad and digestive gland of *Lyonsia hyalina*. No miracidium-mother-sporocyst was found and since the sporocysts produce cercariae, presumably they should be regarded as daughter sporocysts. The tips of all branches are centers of rapid cell-multiplication. They advance by terminal growth into the haemocoelic spaces and ultimately destroy and replace the molluscan tissue (Figs. 8 and 9). On infection of the clam, the miracidium-mother-sporocyst, liberated in the intestine, enters the digestive gland. In mild and presumably recent infections, most of the sporocysts are in the gonad, which seems to be the seat of early involvement, but eventually both gonad and digestive gland are infiltrated and filled with tubules. The diameter of the tubules is variable; sections of very narrow width, 0.02 to 0.03 mm may alternate with short enlarged, distended sections as much as 0.13 mm in diameter. Often longer sections, 0.20 to 0.30 mm long and 0.07 to 0.10 mm in diameter, filled with developing cercariae, are followed by narrow stretches, containing a single row of small germ-balls. The walls of the sporocyst are capable of contraction and distension, but no movement was observed. In certain areas the tegument appears to be bounded by a membrane, lying over a single layer of circular muscle fibers and below this a layer of longitudinal fibers. In other areas, the external membrane appears to be separated from the muscle layers by a narrow band of cytoplasm that contains flattened nuclei. Below the muscle layers there are parenchymal cells and a fluid filled lumen. Certain of these cells have nuclei that stain heavily with haematoxylin and have little cytoplasm. They are more numerous near the tips of the tubules and may be germinal rather than somatic in nature. It appears that these cells undergo cleavage to form spherical germ-balls, 0.01 to 0.05 mm in diameter, composed of a few cells, which separate from the subtegmental layer and become free in the lumen of the tubule. Developmental stages from germ-balls to fully-formed cercariae fill the distended portions of the sporocyst.

Cercariae

In sporocysts, the germ-balls enlarge, become oval, and a constriction by circular muscles demarcates the bilobed tail. In a specimen 0.10 mm long and 0.03 mm wide, the tail stem is well formed and the furcae are about as long as the body. At this stage, they are curved medially and assume the characteristic crossed condition. Fully developed cercariae emerge from the haemocoele into the mantle cavity and pass to the exterior in the excurrent stream through the dorsal siphon. They have the specific gravity of sea water and hang from the furcae which extend upward. Retraction of the furcae, which proceeds in a spiral manner, raises the body and the cercariae are wafted about by currents in the water. They can not swim.

The body is oval to elongate with almost parallel sides, 0.12 to 0.25 mm in length and 0.03 to 0.07 mm in width (Fig. 4). It tends to be circular in cross-section when retracted and flattened on extension. The integument contains spines which decrease in size and number posteriorly. The body wall consists of circular, longitudinal and oblique muscle fibers. The parenchyma contains cystogenous glands, arranged in two longitudinal bands on each side of the body. The cells are irregular in size and shape.

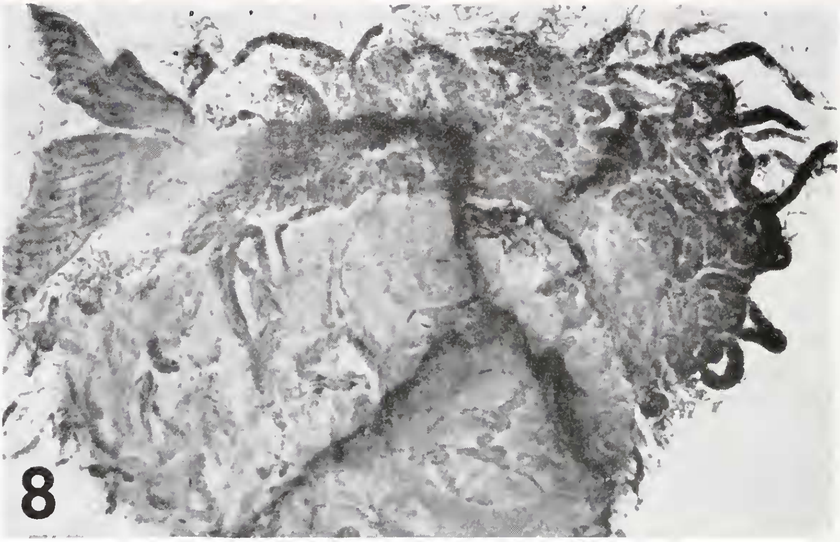
The tail-stem is 0.02 to 0.035 mm long and 0.04 to 0.07 mm wide. It is bilobed, indented posteriorly with a cup-like depression that may function in adhesion. The furcae arise from the anterolateral faces of the tail-stem; their bases are 0.009 to 0.012 mm wide and when fully extended, the furcae are filamentous, ten to fifteen times the length of the body. The furcae have strong circular and longitudinal muscles. The posterior, external surface of each bears papillae and adhesive glands by which they attach to the surface of a fish. When the furca is coiled, the adhesive surface is always external. The posterior half of the tail-stem is filled with similar adhesive glands and when immobilized, as when compressed by slight pressure from a coverglass, the body performs characteristic movements. First, it elongates to full extent, then snaps backward, right or left, until the anterior tip is at the level of the tail-stem, and then again fully extended. The movements occur at intervals of two to five seconds and appear to be the same as those employed in penetration of the skin of a fish.

The so-called "anterior organ" or "penetrating organ" of various authors is 0.05 to 0.06 mm long and 0.035 to 0.045 mm wide. Anteriorly, it opens to the surface by a subterminal pore. From the pore a short duct leads inward to a cupuliform expansion. The posterior portion of the organ is filled with penetration gland cells whose ducts pass forward and open into the cup-like expansion. The posterior half of the organ contains an enormous number of closely packed nuclei. It is suggested that they may be concerned with the formation of the anterior sucker after the regression of the anterior organ. In a discussion of similar "anterior organs" in strigeid and schistosome cercariae, Stunkard (1973) proposed that in these furcocercous cercariae, the organ that functions for penetration of the skin of the next host be designated a "penetrantorium" and the anterior organ of the bucephalid cercaria is a homologous structure.

The pharynx is located near the middle of the body and measures 0.011 to 0.013 mm in diameter. The intestine is mostly post-pharyngeal in extent and the contents stain with neutral red. As the reproductive organs mature, the digestive cecum is pushed forward.

The reproductive organs are represented by a curved mass of deeply staining germinal cells that extends posteriorly from the pharynx to almost the posterior end of the body (Fig. 4). It is the anlage of the gonads and the cirrus sac.

The excretory system begins as separate right and left components in the germ-ball stage with pores at the tips of the furcae. As development of the cercaria is completed, the ducts in the furcae are obliterated and secondary pores are formed at the anterolateral faces of the tail-stem. The loss of the tail-stem leaves a terminal excretory pore. The vesicle extends forward to the level of the gonads and the system is mesostomate with a flame-cell formula of $2[(2+2)+(2+2)]$.



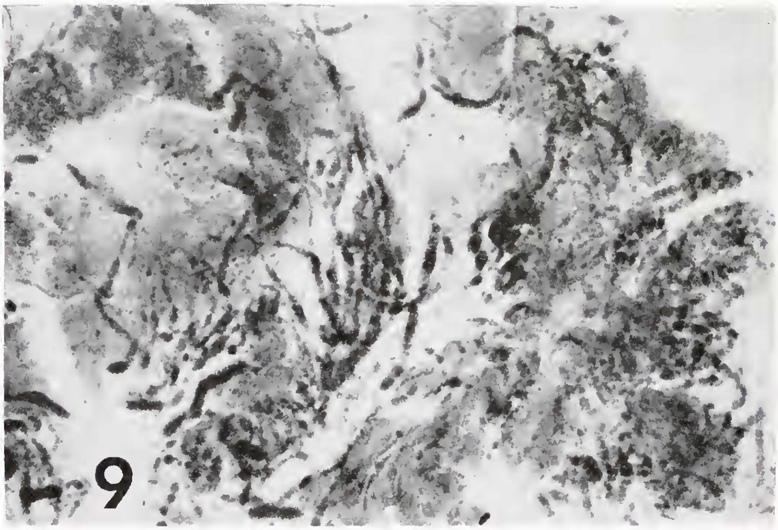


FIGURE 8. Pressed preparation of the tissues of the clam, *Lyonsia hyalina,* infiltrated with branching sporocysts of *Rhipidocotyle* sp.

FIGURE 9. Smear preparation of the tissues of the clam, *Lyonsia hyalina,* infiltrated with branching sporocysts of *Rhipidocotyle* sp.

TAXONOMY

The trematode family Bucephalidae

Prosorhynchoides gracilescens *new combination: validation of* Prosorhynchoides *Dollfus, 1929.* Rudolphi (1819) described three species of bucephalid worms from marine fishes: *Distoma gracilescens* from *Lophius piscatorius* taken at Trieste;

Monostomum galeatum from *Centronotus glaucus* and *Monostomum crucibulum* from *Muraena* spp., both taken at Naples. All were later transferred to *Gasterostomum.* In the period 1890 to 1900, encysted stages of gasterostome metacercariae were found in marine fishes, *Gadus* spp., *Phycis blennioides, Pleuronectes platessa, Belone belone,* and lampreys. These immature worms were generally referred to *G. gracilescens.* Odhner (1905) predicated that the ventral position of the mouth and saccate form of the intestine in gasterostome trematodes were primitive features, retained from a rhabdocoel ancestor, whereas the anterior location of the mouth in other digenetic species was a secondary character and the two groups were descended from different ancestral stocks. He erected two suborders: Gasterostomata for the species in which the mouth was ventral, and Prosostomata for those in which it was at or near the anterior end. Among the dozen or more species of *Gasterostomum* available to him, Odhner recognized two groups, separable on the structure of the adhesive organ, the location of the vitellaria, and the form of the excretory vesicle and of the copulatory organs. In one group the anterior end bore a simple or modified sucker and in the other the anterior end was provided with a solid rostellum or rhynchus. Members of the first group were referred to *Gasterostomum* von Siebold, 1848. Members of the second group were included in a new genus, *Prosorhynchus,* with *Prosorhynchus squamatus* n. sp., from *Cottus scorpius* as type species. Included in the new genus were *Prosorhynchus crucibulum* (Rudolphi, 1819) n. comb. from *Conger conger* and *Prosorhynchus aculeatus* n. sp. for specimens from *Conger conger* that had been assigned to *P. crucibulum* by van Beneden (1870). Odhner (1905) suggested that *Bucephalus cruz* Levinsen, 1881, a furcocercous cercaria from *Modiolaria discors,* may be the larval stage of *P. squamatus.*

The life-cycle of *P. squamatus* was reported by Chubrik (1952, 1966). It was confirmed by Matthews (1973a) who also described the morphology and life-history of *Prosorhynchus crucibulum.* Cercariae of both species developed in branching sporocysts in *Mytilus edulis* and metacercariae encysted in various fishes that served as second intermediate hosts. According to Shulman and Shulman-Albova (1953), *P. squamatus* occurs in several species of arctic fishes, and Chubrik (1966) reported progenetic gravid specimens in intermediate hosts. Matthews noted that measurements of the cercariae of *P. squamatus* agree with those given by Chubrik (1952) and with those given by Cole (1935) for the species from *M. edulis* described by him as *Cercaria mytili* n. sp. All have trilobed tail-stems and the description of *P. squamatus* by Matthews presents no item to differentiate it from *C. mytili.* It is apparent that the two descriptions pertain to the same species. Matthews found eight species of fish serve as second intermediate hosts of *P. crucibulum* but egg production occurred only in the intestine of *Conger conger.*

Prosorhynchus crucibulum is a boreal species which according to Brinkmann (1957) does not occur north of a line from Cape Cod through southern Iceland to Bronnoya, Norway, which is the northern limit of *C. conger.* Manter (1953) had noted differences between specimens of *P. squamatus* and *P. crucibulum* and predicated, p. 194, "In fact, *P. crucibulum* could well be considered in a different genus." Matthews (1973a) listed differences in the cercarial generations; in the morphology of the tail-stem, in shape of the excretory vesicle and in the flame-cell formula. These differences led Stunkard (1974a) to erect a new genus, *Rudolphinus,* with *Rudolphinus crucibulum* as the type species.

Diesing (1858) made a revision of the Trematoda. In it he erected the genus *Rhipidocotyle* to contain *Gasterostomum gracilescens* (Rudolphi, 1819) and *Gasterostomum minimum* Wagener, 1852, but he did not designate a type species. Stiles and Hassall (1908) tentatively selected *G. gracilescens* as type but Nicoll (1914) observed that the name *Rhipidocotyle* referred to a fan-shaped hood or cowl that surmounted the anterior sucker of *G. minimum,* but is not present in *G. gracilescens.* Accordingly, *Rhipidocotyle minima* was recognized as type of *Rhipidocotyle.* Nicoll (1914) adopted the family name Bucephalidae Poche, 1907 (syn. Gasterostomidae Braun, 1883) and erected two new subfamilies: Prosorhynchinae, with the single genus *Prosorhynchus* Odhner, 1905 and Bucephalinae with three genera: *Bucephalus* von Baer, 1826; *Bucephalopsis* Diesing, 1858; and Rhipidocotyle Diesing, 1858. The type material of *Gasterostemum galeatum* (Rudolphi, 1819) Stossich, 1858 and of *G. minimum* Wagener, 1852 from the Berlin Museum was studied by Eckmann (1932) who reported that *G. minimum* is identical with *Monostomum galeatum* Rudolphi, 1819. Consequently, *Rhipidocotyle galeata* (Rudolphi, 1819) Eckmann, 1932 is recognized as the type of the genus.

The species, *Distoma gracilescens* Rudolphi (1819 has had a resilient history; it was transferred to *Gasterostomum* by Wagener (1852), to *Rhipidocotyle* by Diesing (1858), to *Prosorhynchus* by Lebour (1912), to *Bucephalopsis* by Nicoll (1914), and to *Bucephaloides* by Hopkins (1954). According to Srivastava and Chauhan (1972), *Bucephaloides* Hopkins is a junior synonym of *Prosorhynchoides* Dollfus, 1929. Nagaty (1937) had recognized the validity of *Prosorhynchoides* Dollfus, 1929 with *Prosorhynchoides ovatus* (Linton, 1900) as type species. Srivastava and Chauhan (1972) transferred all the species previously included in *Bucephaloides* to *Prosorhynchoides. Distoma gracilescens* Rudolphi, 1819 thus becomes *Prosorhynchoides gracilescens* (Rudolphi, 1819) new combination.

The life-cycle of the species was reported, under the designation, *Bucephaloides gracilescens* (Rudolphi, 1819) Hopkins, 1954 by Matthews (1974). The sporocysts and cercariae were found in *Abra alba* from the Firth of Clyde, off the Isle Cumbrae, at depths of 40 fathoms and from the Lang channel at depths of 10 to 20 fathoms. About 15% of the clams were infected. Matthews reported, p. 3, "a thick solution of 'Polycel' was found useful in slowing down movements of cercariae and miracidia." This statement is surprising since gasterostome cercariae are relatively inactive and there was no other mention of the miracidium. Indeed, the miracidium has not been described for any marine gasterostome. Moreover, on p. 6 Matthews stated, "The flame cells divide as shown, the one posterior to the pharynx giving rise to that part of the flame system which passes into the posterior half of the body." And on p. 6, "Further division of the flame cells in *B. gracilescens* beyond the twelve flame cell stage have not been followed." These statements are obviously based on the unsupported presumption of Faust (1919), who accounted for the increase in flame-cells by division. During development of cercariae, additional flame-cells appear in a regular sequence and pattern, but not by division of previous cells. The cercaria is very similar and probably identical with *Cercaria syndosmyae* Lebour, 1912, described from the same host and the same location with an infection rate of about 20%. Matthews noted, p. 11, "The incidence and geographic location of this species is almost identical with that of *B. gracilescens* in this host." Matthews distinguished between the two species since Miss Lebour (1912) did not describe the sporocyst and in *C. syndosmyae* the figure shows the pharynx located at the level of the anterior half of the intestine. Neither of these feaures appears relevant. Miss Lebour figured only the tip of a branch of the sporocyst and the location of the pharynx with reference to the intestine is too variable to be species specific. The metacercaria has been recorded from nine different species of ganoid fishes. Its occurrence in the nervous system especially, encysted on cranial and spinal nerves of various fishes was noted by Munro (1785), and described by Olsson (1868), Johnstone (1905), and Lebour (1908, 1912). Matthews (1974) reported the development of the cercaria and the effect on the host. The metacercaria was linked with the adult on the basis of comparative morphology and ecology of hosts.

Rhipidocotyle campanula *(Dujardin, 1845) new combination: second freshwater bucephalid in Europe.* The taxonomy of the bucephalid trematodes is complicated because of unsupported and unwarranted presumptions concerning relations between larval and adult stages. Furcocercous cercariae from the European freshwater clams, *Anodonta mutabilis* and *Unio pictorum*, were described by von Baer (1827) as *Bucephalus polymorphus.* The larvae swim with the furcae in advance which prompted the generic name, *Bucephalus.* A graphic account of the swimming behavior was given by Badcock (1875) who reported, p. 142, that the cercariae "presented a beautiful sight of transparent creatures flying like eagles through the water, the wing-like appendages spreading out to an enormous length, and constantly in motion, with a general upward tendency. They never attached themselves to any object, but always swam freely, and were neither seen to feed upon other creatures, or to be objects of attack." Mature worms from the intestine of *Perca fluviatilis* and *Lucioperca* spp. were described by von Siebold (1848) as *Gasterostomum fimbriatum.* From morphological agreement, von Siebold predicated that these worms represented the adult stage of *B. polymorphus.* The probable identity was supported by Wagener (1858), Giard (1874), Ziegler (1883), and until recently all bucephalid parasites from European freshwater hosts were referred to a single species. Despite the recognized synonymy with *Bucephalus*, new species as described were assigned to *Gasterostomum* and a dozen or more were recognized by the end of the 19th century.

A new digenetic trematode was described by Dujardin (1845) under the name, *Distoma campanula.* He reported, p. 435, "J'ai trouvé à Rennes, dans l'intestin du brochet (*Esox lucius*) des petits distomes, moitié plus petits que le *Distoma nodulosum,* quoique adultes, et contenant aussi des oeufs plus petits de 0 mm 028; ils en différent en outre par leur ventouse ventral, beaucoup plus petite; la ventouse antèrieure est entourée à un large bord, finement plissé, en forme de cloche, mais non lobé; le tégument est parsemé de petites épines."

The species, *Distoma campanula* Dujardin, 1845 was redescribed and figured by Wedl (1858). The specimens were from the intestine of *Esox lucius.* The worms were about 1.00 mm long, 0.25 mm wide. The anterior sucker had two small lateral projections and was provided with a strong layer of muscle-fibers which extended in a concentric fashion on the ventral side and formed a ridge on the dorsal side of the anterior sucker. The pharynx was situated in the posterior half of the body and measured 0.06 mm in diameter. The vitellaria consisted of slight extensions in the anterior part of the body but were not shown in the figure. The uterus was massive, and filled with eggs that obscured the gonads. The eggs measured 0.028 mm in length. In the posterior part of the body Wedl figured an "oblonges Organ", apparently the cirrus-sac, but the genital pore was not observed. The tegument was provided with spines which diminished in size posteriorly. The redescription by Wedl clearly established the validity of *Distoma campanula* Dujardin.

Wagener (1852) reported *Gasterostomum fimbriatum* von Siebold, 1848 from the intestine of *Esox lucius* taken in the region of Berlin. He described the tentacles, which he found to number five, and observed that they may be extended and retracted. In the latter condition, they may not be visible. Additional descriptive data and figures were provided by Wagener in (1857). He (1858) suppressed *D. campanula* as a synonym of *G. fimbriatum* and recognized the identity of the latter species and *B. polymorphus*. The identity of *B. polymorphus* and *G. fimbriatum* was based on morphological similarity and the belief that there was only one species of bucephalid trematodes in the freshwater fishes of Europe. But there was no experimental evidence to support such a belief or for the identity of the sporocysts and cercariae that were found in the species of *Anodonta* and *Unio*. There was no assurance that *B. polymorphus* pertained to a single species. Furthermore, if *D. campanula* and *G. fimbriatum* are identical, the valid name of the species is *campanula*, not *fimbriatum*. However, later authors accepted the identity of the two forms and recognized *B. polymorphus* as the single bucephalid species in Europe.

Ziegler (1883) found mature bucephalid worms in the intestine of *Esox lucius* from the Ill River near Strassburg and sporocysts and cercariae in *Anodonta mutabilis*. The adults were described as *G. fimbriatum* and the cercariae as *B. polymorphus*. Specimens of *Leuciscus erythrophthalmus* were exposed to the cercariae and, on dissection later, harbored encysted metacercariae. The experiments lacked proper controls, but it is possible that Ziegler discovered the life-cycle of the species he studied. Discussing the cercariae, he declared that the anterior end does not bear a mouth as described by Pagenstecher (1857) but instead it consists of an "abgegrenster Komplex von Drüsen und Parenchymzellen (Fig. 9)". He referred the cercariae to *B. polymorphus* but noted in a footnote, p. 541, "Wenn Baer (No. 2, p. 575) grössere Dimensionen angiebt, so kann darin kaum specifischer Unterschied gesehen werden." Ziegler compared his adult specimens with descriptions of *G. fimbriatum* as given by Wagener (1852, 1857, 1858). He recalled that Wagener (1857) had described fingerlike processes at the anterior end of the body, "fünf gablige hohle Fühler oder Rüssel; sie könnten durch fünf musculöse Stränge eingestülpt werden, die von im Grunde des Saugnapfes verlaufenden scheiden umhüllt seien." Referring to the retractile tentacles, Ziegler stated, p. 542, "Ich habe dies Gebilde weder bei alten noch bei jungen Thieren je gesehen; da nun alle weiteren Angaben Wagener's vollkommen auf die mir vorliegende Form passen, so ist es mir unwahrscheinlich, dass seine

Darstellungen auf eine andere Species sich beziehen; ich bin eher geneigt zu glauben, dass die "Fimbrien" nur unter selten, vielleicht abnormen Verhältnissen auftreten und werde bei der Beschreibung des vorderen Saugnapfes die Entstehung derselben zu erklären suchen." Nothwithstanding this declaration, in a footnote on the same page, Ziegler predicated, "Wenn nachgewissen würde, dass die Fimbrien bei *Gasterostomum fimbriatum* eine normale und konstante Erscheinung sind, so müsste die im Flussgebiet der Ill gefundene und von mir beschriebene Form als eine neue Species angesehen werden und könnte *Gasterostomum illense* genannt werden." Although Ziegler recognized that the specimens from *E. lucius* might not be referrable to *G. fimbriatum,* in his description and figures he included them in that species.

Lühe (1909) described *B. polymorphus* von Baer and listed *G. fimbriatum* von Siebold as a synonym. The adult worms were from *Esox lucius, Perca fluviatilis, Lioperca lucioperca,* and *Lota lota;* the cercariae developed in various Unionidae. But his figure of *B. polymorphus* was clearly of the species described by Dujardin (1845), Wedl (1858) and Ziegler (1883). It can not be included in *Bucephalus* and clearly is a member of the genus *Rhipidocotyle.* Whether or not *Gasterostomum* is a synonym of *Bucephalus* is still uncertain.

The presence of a single bucephalid species in the freshwater hosts in Europe was accepted by Eckmann (1932), Nagaty (1937), Dawes (1946) and Hopkins (1954). But the existence of two distinct species is now recognized. Kowal (1949) found two species which he described as *Bucephalus polymorphus* and *Bucephalus markewitschi.* Vejnar (1956) identified the species without tentacles as *Rhipidocotyle ilense* (Ziegler, 1883) new combination. Kozicka (1959) admitted the identity of *Distoma campanula* Dujardin, 1845 and *Rhipidocotyle illense* (Ziegler, 1883) Vejnar, 1956. Yamaguti (1971) declared that *Rhipidocotyle illense* (Ziegler, 1883) Dyk, 1954 is a synonym of *Distoma campanula* Dujardin, 1845. The synonymy is well established and the species without tentacles is designated by its correct name, *Rhipidocotyle campanula* (Dujardin, 1845) new comb.

Lacaze-Duthiers (1854) described sporocysts and cercariae from the oyster, *Ostrea edulis,* taken at Mahon in the Balearis Isles and from *Cardium rusticum,* taken in l'étang du Thau near Cette on the south coast of France. There is no assurance that the parasites from the two hosts and two locations are specifically identical. Indeed, there is strong probability that two distinct species are involved. Environmental conditions at the two locations are very different. *Ostrea edulis,* the type host, is an oceanic species and the water at Mahon has high salinity; whereas at l'étang du Thau, the water had a reduced and varying salinity and an increased and varying temperature. The name *C. rusticum* has been suppressed as a synonym of *Cardium glaucus* Bruguiere, 1789 and as a subspecies of *Cardium edule.* There is a question concerning the identity of *C. edule.* Discussing Mediterranean species, Mars (1966) declared, p. 300, "Rappelons que nous n'avons jamais recontré l'espèce atlantique *Cardium edule,* ni sur le littoral, ni dans les gisements quarternaires, ni dans aucun étang de nos régions."

Referring to *B. haimeanus,* Matthews (1973b) observed, p. 341, "In addition to *Ostrea edulis* L., the type host, it has been found in eight other species of bivalves namely, *Cardium rusticum* L., by Lacaze-Duthiers (1854); *C. edule* L. by Huet (1888), Johnstone, (1905), Pelseneer (1906), Lebour (1907) and

James and Bowers (1967); *Spisula solida* (L.) by Huet (1888); *Venerupois decussata* (L.) and *V. pullastra* (Montagu) by Vaullegeard (1894); *V. aurea* (Gmelin) by Andreu (1949); *Abra alba* (Wood) by Pelseneer (1906) and *Crossostrea virginica* by Tennent (1905, 1906, 1909). All of these, with the exception of *C. virginica,* occur in Europe. *C. virginica* inhabits coastal waters off the east coast of North America." Lebour (1912) reported that in Britain *B. haimaeanus* has been observed only in the cockle, *Cardium edule,* but always in the vicinity of oyster beds. The fact that in the Plymouth area the parasite infected only cockles but not oysters in adjacent areas, is additional evidence that the species in *C. edule* is not *B. haimeanus* Lacaze-Duthiers. Matthews (1973b) reported the life-cycle of *B. haimeanus* from *C. edule.* He predicated, p. 348, "*B. haimeanus* is associated with the fauna of the estuarine habitat, the first intermediate host being *Cardium edule* and the second intermediate host *Pomatoschistus microns.*" The cockles were taken from Dovey Estuary, Cardigan Bay, Wales. Metacercariae were obtained experimentally in *Pomatoschistus microns* and *Pleuronectes platessa.* The metacercaria was assigned to *Bucephalus minimus* Stossich, 1887 on the basis of comparative morphology and ecology of hosts. Adult specimens were described by Stossich (1887) from *Morone labrax* from the Mediterranean and by Nicoll (1914) from the same host taken in the Irish Sea. Matthews observed, p. 349, "In *B. haimeanus* the tentacles are much reduced in comparison with *B. polymorphus* suggesting an evolutionary link with the marine *Rhipidocotyle* through the estuarine habitat." The report by Matthews confirms the conviction that the parasite from *C. edule* is not *B. haimeanus.*

The first report of an American bucephalid was made by McCrady (1874) who described branching sporocysts and gasterostome cercariae from the oyster, *Crossostrea viriginica* at Charleston, South Carolina. The sporocysts were rigid, not contractile, and apparently resembled those of *B. polymorphus* von Baer, 1827, a freshwater species in Europe rather than those of *B. haimeanus* Lacaze-Duthiers, 1854, a parasite of the European oyster. The furcae were long and provided with pointed cells along their length. The parasite was described as a new species, *Bucephalus cuculus.* Bucephalid sporocysts and cercariae were reported from New Jersey by Nelson (1890, 1903, 1915). *Bucephalus cuculus* was reported from oysters at the Gulf Biological Station, Cameron, Louisiana by Glaser (1904). Tennent (1905, 1906, 1909) identified the parasite of oysters at Charleston, S. Carolina as *B. haimeanus.* He presumed that the cercariae were identical with the metacercariae in *M. menidia* and the adults from *Strongylura marina* that had been assigned to *Gasterostomum gracilescens* by Linton (1905). None of these allocations were correct. The life-cycle had not been demonstrated for any American marine gasterostome until the report by Stunkard (1974b).

The life-cycles of three North American freshwater bucephalids have been elucidated. They are *Rhipidocotyle papillosa* (Woodhead, 1929) Eckmann, 1932 by Woodhead (1929); *Bucephalus elegans* Woodhead, 1929 by Woodhead (1930); and *Rhipidocotyle septpapillata* Krull, 1934 by Krull (1934) and Kniskern (1952a, b).

The other experimentally-proved life-cycle was done by Howell (1966). He showed that sporocyst and cercarial generations of *Bucephalus longicornutus* (Manter, 1954) occur in the New Zealand mud-oyster, *Ostrea lutaris* Hutton,

1873. Metacercariae were recovered from *Tripteron* spp. and *Acanthoclinus quadrïdactylus.* Adults occur in *Scorpaena cardinalis* Richardson.

At present, a challenging problem in the family Bucephalidae is the discovery of the life-cycles, secondary and definitive hosts of *B. haimeanus,* from the European oyster and of *B. cuculus* from the American oyster. It is distressing to realize that this situation has remained unresolved for more than a century.

SUMMARY

The life-cycles of *Rhipidocotyle transversale* Chandler, 1935 and *Rhipidoctoyle lintoni* Hopkins, 1954 have been determined. Both species employ the same intermediate and final hosts; metacercariae in silversides, *Menidia menidia*, and adults in gars, *Stronglyura marina.* Branching sporocysts and furcocercous cercariae occupy the gonad and digestive gland of *Lyonsia hyalina* but can not be assigned with certainty to either or both species, because identifying morphological features are not yet developed. Adult and metacercarial stages of both parasites occur in the same host-species and it is probable that the larval stages of both are in *L. hyalina.*

Recent studies on life-cycles of bucephalid trematodes demand revision of certain taxonomic allocations. The status of *Distoma gracilescens, Monostomum galeatum,* and *Monostomum crucibulum,* all described by Rudolphi (1819), are reviewed. *Distoma gracilescens* was transferred to *Gasterostomum* by Wagener (1852), to *Rhipidocotyle* by Diesing (1858), to *Prosorhynchus* by Lebour (1912), to *Bucephalopsis* by Nicoll (1914), and to *Bucephaloides* by Hopkins (1954). But *Bucephaloides* Hopkins, 1954 is a junior synonym of *Prosorhynchoides* Dollfus, 1929 and *Distoma gracilescens* becomes *Prosorhynchoides gracilescens* (Rudolphi, 1819) new combination. The life-cycle of the species was reported by Matthews (1974). *Monostomum galeatum* was transferred to *Gasterostomum* by Stossich (1898) and declared identical with *Gasterostomum minimum* Wagener, 1852 by Eckmann (1932). Accordingly, it becomes the type-species of *Rhipidocotyle* Diesing, 1858. *Monostomum crucibulum* was transferred to *Gasterostomum* by Gervais and Beneden (1859), and to *Prosorhynchus* by Odhner (1905). The life-cycle was reported by Matthews (1973a), and Stunkard (1974a) named it type of a new genus, *Rudolphinus.*

The status of *Bucephalus polyphemus* von Baer, 1827 and *Gasterostomum fimbriatum* von Siebold, 1848, long regarded as specifically identical and the only bucephalid species in freshwater hosts in Europe, is equivocal. *Bucephalus polymorphus* is the name of a cercaria whose adult stage is yet to be disclosed and the larval stages of *G. fimbriatum* are unknown.

Dujardin (1845) described small distomes from the intestine of *Esox lucius* taken at Rennes, France, as *Distoma campanula.* The species was redescribed and figured by Wedl (1858). *Distomum fimbriatum* von Siebold (1848) was redescribed by Wagener (1852, 1857, 1859). He (1858) suppressed *D. campanula* as a synonym of *G. fibriatum,* and recognized the latter species as the adult of *B. polymorphus.* Ziegler (1883) described mature bucephalid worms from *E. lucius* taken in the Ill River near Strassburg as *G. fimbriatum* and larval stages from *Anodonta mutabilis* as *B. polymorphus.* In a footnote, he predicated that if his specimens did not prove to be identical with *G. fimbriatum,* they should be regarded as a new species, *Gasterostomum illense.* A second bucephalid species

from freshwater fishes in Europe was described by Kowal (1949), Vejnar (1956), and Kozicka (1959). It was designated *Rhipidocotyle illense* (Ziegler, 1883) Vejnar, 1956 by Kozicka (1959). Comparison with descriptions of *Distoma campanula* show such agreement that the two must be regarded as identical and the name of the species is *Rhipidocotyle campanula* (Dujardin, 1845) new combination.

Bucephalus haimeanus was described by Lacaze-Duthiers (1845) from cercariae found in oysters. *Ostrea edulis,* at Mahon in the Balearic Isles and in *Cardium edule* taken in l'étang du Thau near Cette on the south coast of France. There is no evidence that the cercariae from the two hosts and two locations are identical. Lebour (1912) reported *Cercaria haimeanus* from *C. edule* at Plymouth, but no infection in oysters. Matthews reported that cercariae from *C. edule,* identified as *Cercaria haimeanus,* developed into adults identical with *Bucephalus minimum* Stossich, 1887. The adult stages of the cercariate from European oysters, and of another species, *Bucephalus cuculus,* parasitic in the American oyster, *Crossostrea virginica,* are still unknown after more than one-hundred years.

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